

The influence of ionizing radiation on differentiation and function of osteoclasts*

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Introduction

Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease that has a prevalence of about 1% and is more frequent in women than in men. It is usually accompanied by a symmetric Polyarthritits (PA) with synovial infiltration and invasion of inflammatory immune cells [1]. This often leads to local and systemic cartilage and bone destruction by osteoclasts (OC) as well as inhibition of bone formation by osteoblasts [2]. While there are effective treatments for RA and other degenerative musculoskeletal diseases there are patients that do not respond to these therapies or have to abort treatments due to health related problems. In this case a treatment with low dose-radiotherapy (LD-RT) can be helpful to modulate the immune system and thus lead to an anti-inflammatory effect [3]. However the underlying mechanisms are only fragmentarily understood. This is why we have examined the effects of LD-RT on osteoclast progenitor cells from healthy donors and on the differentiation of osteoclasts derived from the bone marrow of *hTNF-α* tg mice, an accredited inflammatory mouse model for RA [4].

Material and Methods

Bone marrow from the long bones of 5-6 week old *hTNF-α* tg mice was isolated and stimulated with media containing 10ng M-CSF and 50ng RANK-L. 24h after seeding cells were treated with various doses of X-rays. TRAP-Stain (Sigma) positive cells with at least 3 nuclei were counted as OCs. Decalcified paw sections from locally irradiated animals were analysed using the Osteomeasure™ software (Osteometrics). Human OCs were generated from buffycoats of healthy donors (blood donor service Frankfurt/Main). Differentiation of human OCs from monocytes was initiated by adding RANKL (45 ng/ml) and M-CSF (25 ng/ml). The resorbing activity of OCs was detected on bovine bone slices via toluidin blue staining. Irradiation was performed with X-rays (1Gy/min).

Results

The numbers of differentiated *hTNF-α* tg bone marrow-derived OCs were significantly reduced after administration of X-ray doses of 0.05 – 2.0 Gy (Fig.1A).

Investigation of decalcified, paraffin embedded paw sections revealed a reduction in OCs/mm² in irradiated paws of *hTNF-α* tg mice in comparison to non-irradiated control animals (Fig. 1B). As a functional analysis, the resorptive activity of human OCs after irradiation was determined by calculating the area of resorption pits

(Fig.1C). OCs resorbed significantly less bone after exposure to both, low and high dose of X-rays.

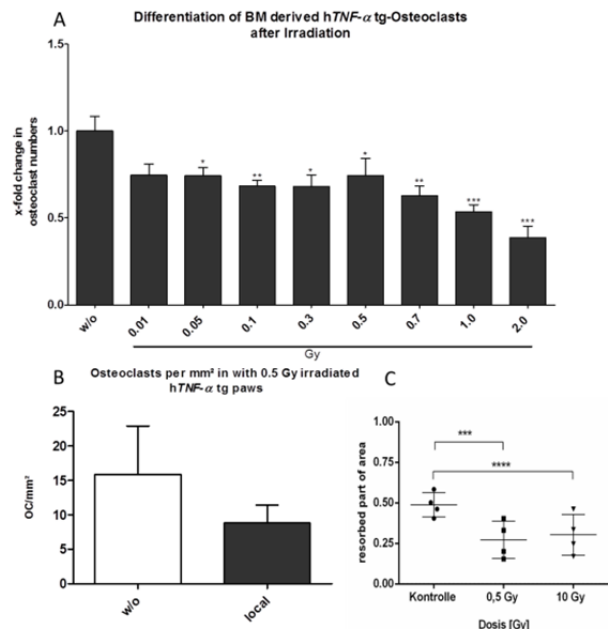


Figure 1: A) Murine bone marrow-derived OCs after treatment with various doses of X-rays. The x-fold change in relation to non-irradiated control cells is displayed. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ B) OCs/mm² in TRAP stained paw sections of locally irradiated *hTNF-α* tg mice as well as control animals (w/o). C) Resorbing activity of human OCs quantified by resorption pits via toluidin blue staining (N=2, n=4).

Conclusion

We suggest a useful supportive role of LD-RT for patients suffering from RA and other degenerative musculoskeletal and inflammatory diseases as X-rays have a considerably decelerating effect on osteoclast differentiation and impair their function. This was not only observed *in vitro/ex vivo*, but also *in vivo* in mice with established PA. This provides an indication that, especially in a pre-existing inflammatory state, X-rays are capable of attenuating the bone-destruction by OCs.

References

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